

University of Groningen

## Gene expression in Hodgkin lymphoma. The role of miR-155 in normal and malignant B cells

Kluiver, Joost Laurens

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2006

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Kluiver, J. L. (2006). *Gene expression in Hodgkin lymphoma. The role of miR-155 in normal and malignant B cells*. s.n.

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

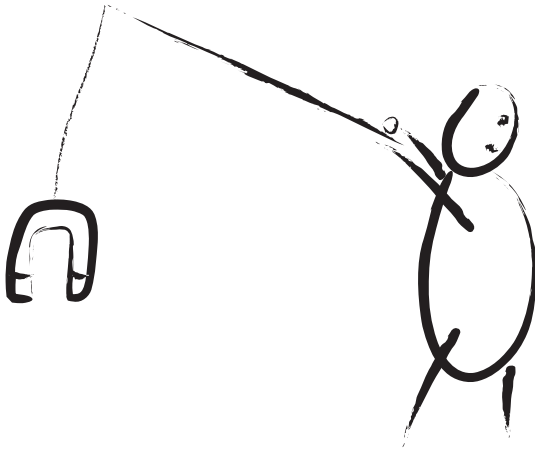
### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

# *CHAPTER 4*

*microRNA biogenesis and functioning*



*Joost Kluiver*

## Introduction

**M**icroRNAs (miRNAs) are now recognized as an important class of small RNA molecules that can posttranscriptionally downregulate the expression of genes bearing complementary target sequences. A miRNA is currently defined as a small single stranded RNA of ~22 nt that is generated by RNase III type enzymes from a hairpin present in a longer endogenous transcript<sup>1</sup>. It is thought that ~2-3% of all genes have a miRNA function indicating that in human, there are at least 800 miRNA genes<sup>2-5</sup>. At this moment, 332 miRNA are present in the miRNA database<sup>6</sup>.

## Biogenesis

**B**iogenesis of a miRNA starts with the transcription of a longer primary transcript (pri-miRNA) by RNA polymerase II<sup>7,8</sup>. The pri-miRNA contains a hairpin structure that can be recognized and cleaved by the microprocessor complex which consists of the RNase III enzyme DROSHA and the double stranded RNA binding protein DGCR8<sup>9-13</sup>. The resulting ~65 nt precursor miRNA (pre-miRNA) is exported to the cytoplasm by the RAN-dependent nuclear transport receptor exportin-5<sup>14-17</sup> for further processing into a ~22 nt miRNA duplex by the RNase III enzyme DICER<sup>18-21</sup> and its partner the human immunodeficiency virus transactivating response RNA-binding protein (TRBP)<sup>22,23</sup>. Based on the stability at the 5' end of the miRNA duplex, one strand will be degraded while the other remains associated with DICER to form an active RNA-induced silencing complex (RISC)<sup>24-26</sup>. The RISC complex generally binds to complementary sites in the 3' UTR of target genes and acts by either translational inhibition or by mRNA cleavage. The complex can contain several other components besides DICER, i.e. TRBP, Argonaute family proteins (Ago)<sup>27</sup>, PACT<sup>28</sup>, MOV10<sup>29</sup> and TNRC6B<sup>29</sup>. TRBP, Ago2 and PACT have been shown to be necessary for miRNA directed target mRNA degradation<sup>22,27,28</sup>. In general, the exact functions of most of the RISC components are not fully understood. It is currently thought that in animals the RISC complex acts mainly by inhibition of translation and to a lesser extent by degradation of mRNA target transcripts. In recent studies, so-called processing bodies or P-bodies were demonstrated to be involved in miRNA directed translational inhibition<sup>30-33</sup>. Sequestration of target mRNAs into P-bodies is supposed to make them unavailable to the translational machinery.

## Functioning

To date, the role of most miRNA is largely unknown. To obtain a better insight into miRNA function, much effort has been put in the computational identification of miRNA targets using various algorithms. The algorithms used in these prediction programs rely mainly on criteria like, base pairing rules between mRNA and miRNA target sites, localization of the target site in the 3' UTR, conservation of the target site in related genomes and free energy of binding between RNA:RNA duplexes<sup>33-45</sup>. In addition, the 5' end of miRNA, i.e. nt 2-8 or "the miRNA seed", has been shown to be very important in miRNA target gene recognition<sup>46-48</sup>. Most algorithms use this seed sequence to search for complementary sequences in the 3' UTR of genes. A recent report also takes into account miRNA target site accessibility based on the secondary structure of the target mRNA<sup>43</sup>. These computational studies have shown that miRNA might regulate up to 200 gene targets that can have very diverse functions, e.g. transcription factors, secreted factors, receptors and transporters. However, these are all predictions with a substantial false positive rate, and only very few predicted miRNA targets have been verified so far. One should also take into account that the miRNA and target mRNA are not always expressed in the same cell. Thus, miRNA can have different targets in different cell types.

## microRNA in human biology and disease

Some reports have now shown a role for miRNA in various biological processes, especially in differentiation and development. For instance, miR-1 was shown to play a role in the development of heart and skeletal muscle, miR-134 regulates dendritic spine development, miR-143 is proposed to regulate adipocyte differentiation and miR-196 was shown to play a role in limb development<sup>49-53</sup>.

Several diseases may also have a miRNA related origin. A mutation in the 3'UTR of the *SLITRK1* gene in 2 patients with Tourette's syndrome enhanced the miR-189 directed translational inhibition of *SLITRK1* mRNA<sup>54</sup>. Together with the demonstrated *SLITRK1* and miR-189 expression patterns in developing brain, this suggested a role for miR-189 in Tourette's syndrome. Two studies have provided evidence for a role of the liver specific miR-122 in metabolic diseases by administering specific inhibitors to normal mice<sup>55,56</sup>. In both studies a significant decrease in plasma cholesterol levels was achieved after inhibitor treatment. Similar effects could be shown in a diet-induced obesity mouse model with also a significant improvement in liver steatosis. These studies suggest that miR-122 plays

a role in the regulation of cholesterol levels and might be an attractive therapeutic target for high cholesterol related diseases. Many viruses, including herpesviruses, polyomaviruses and retroviruses were shown to encode miRNA<sup>57-67</sup>. Whether these viral miRNA are directed against viral target genes, host target genes or both is currently unknown. In the case of EBV, 17 miRNA were shown to be expressed depending on the type of infection<sup>60,66</sup>. These miRNAs were shown to be more conserved than the total viral genome in comparison to rhesus lymphocryptovirus, an equivalent to the human EBV<sup>66</sup>. The extensive miRNA conservation between related viruses suggests that their targets predominantly are of cellular and not of viral origin. However, a recent study did show that the EBV miR-BART2 regulates the expression of the viral *BALF5* gene<sup>60</sup>.

A large body of evidence has accumulated indicating an important role of miRNA in oncogenesis<sup>33,68-75</sup>. miRNAs were shown to be frequently located at fragile sites or at genomic sites that are often altered in cancer<sup>76</sup>. Some specific miRNAs, that were shown to be underexpressed, mapped to regions that are frequently deleted<sup>77,78</sup> and may thus be potential tumor suppressor miRNAs. Other miRNAs were found to be overexpressed and located at regions frequently amplified in cancer and could therefore be considered as oncogenic miRNA (or onco-miRNA)<sup>79,80</sup>. Finally, miRNA expression profiling was reported to allow a better definition of specific subtypes of cancer than an expression profile of 16,000 genes<sup>81</sup>. Several studies have now used miRNA profiling to identify deregulated miRNA expression in various types of cancer<sup>82-87</sup>.